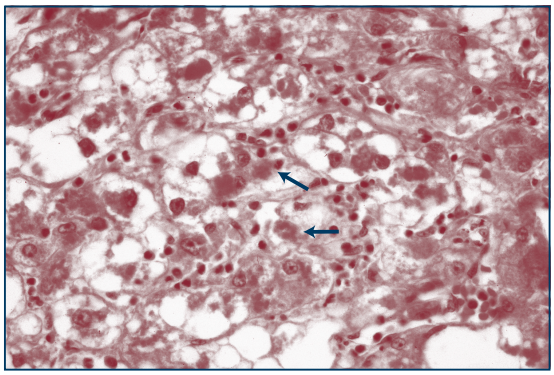
Alcoholic Hepatitis

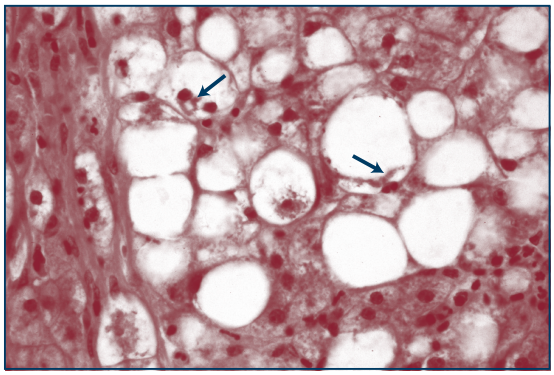


High Alcohol Intake

AST>ALT

BMI<25

Nonalcoholic Steatohepatitis



Low to No Alcohol Intake

ALT>AST

BMI>30

Fat can accumulate in the liver and cause inflammation and injury in people who consume large amounts of alcohol or in those who are overweight (body mass index or BMI≥25) or obese (BMI≥30) and consume little to no alcohol. The former condition is known as alcoholic hepatitis and the latter as nonalcoholic steatohepatitis or NASH. While the triggers differ, liver biopsies from patients with these two conditions (shown) both reveal fat accumulation (round transparent structures) and similar signs of injury such as Mallory bodies (see arrows) and neutrophils (immune cells). However, the conditions are distinguished clinically by the relative levels of two enzymes released from the liver (AST and ALT). Photos: Dr. Zachary Goodman, Division of Hepatic Pathology, Armed Forces Institute of Pathology.

CHAPTER 7: FATTY LIVER DISEASE

INTRODUCTION AND BACKGROUND

Fatty liver disease occurs in two major forms—alcoholic and nonalcoholic. Both forms are marked by accumulation of fat in the liver with variable amounts of liver injury, inflammation, and fibrosis. The spectrum of fatty liver disease ranges from simple steatosis (considered benign and non-progressive), to steatohepatitis (fatty liver with liver cell injury and inflammation), to progressive hepatic fibrosis and cirrhosis. Clinical and histological features are not reliable in separating alcoholic from nonalcoholic forms of fatty liver disease, with the separation being based largely on patient history of alcohol intake.

Both forms of fatty liver disease are common. Alcoholic liver disease affects approximately 1 percent of the adult U.S. population and accounts for half of deaths due to cirrhosis. Nonalcoholic fatty liver disease is the most common reason for liver test abnormalities in the general population and may be present in as many as a quarter of adult Americans. Nonalcoholic liver disease, when accompanied by liver injury, hepatocellular necrosis, inflammation, and fibrosis, is referred to as nonalcoholic steatohepatitis (NASH). NASH accounts for about 10 percent of newly diagnosed cases of chronic liver disease and is believed to be the underlying etiology in at least 10 percent of cases of cirrhosis. Indeed, a large proportion of so-called cryptogenic cirrhosis, which accounts for 9 percent of liver transplants in adults, may be due to end-stage NASH, at which point the typical histologic features (i.e., florid amounts of fat and ballooning degradation) may have disappeared.

NASH is associated strongly with obesity and type 2 diabetes, conditions that have been increasing markedly in the U.S. population in the previous two decades. In similar fashion, NASH is also increasing in frequency in the United States. While NASH is usually identified in adults with obesity and underlying diabetes, it can also occur in normal weight persons without diabetes or hyperlipidemia and has been described in children as well.

There are also secondary forms of nonalcoholic fatty liver disease that are caused by other known factors, such as jejunoileal bypass surgery, short gut syndrome, certain medications (methotrexate, amiodarone), and total parenteral nutrition (TPN). Among these, TPN-associated liver disease is the most common and accounts for an increasing proportion of liver transplants done in children.

The fundamental causes and mechanisms of injury in alcoholic and nonalcoholic fatty liver disease have been only partially defined. The hepatic steatosis is due to accumulation of lipids, predominantly triglycerides, within hepatocytes due to variable combinations of excess lipid synthesis and uptake with altered intermediate metabolism and secretion. In alcoholic liver disease, the accumulation of fat is believed to be due to effects of alcohol on the redox state (the balance of oxidizing and reducing reactions in the cell), cytokines, and transcription factors that control rates of fatty acid synthesis and oxidation. In NASH, the cause of fat accumulation is less well

defined, but its association with obesity and diabetes suggests that it is a component of the dysmetabolic syndrome. The majority of patients with NASH have insulin resistance, which may account for the altered lipid metabolism and perhaps cell injury.

In both alcoholic and nonalcoholic liver disease, a central issue is the progression of simple steatosis (fatty liver) to steatohepatitis (NASH or alcoholic hepatitis). Excessive alcohol intake leads to steatosis, but is reversible with abstinence, and even continued intake does not necessarily cause liver cell injury or alcoholic hepatitis. Similarly, obesity and excessive caloric intake commonly cause hepatic steatosis, but do not necessarily lead to steatohepatitis and significant liver injury. Thus, in both conditions the cause of the "second hit" required for progression of steatosis ("first hit") to steatohepatitis is unknown, but is critical for understanding the pathogenesis of these diseases and designing effective approaches to prevention or therapies.

RECENT RESEARCH ADVANCES

Important research advances have been made in understanding and managing alcoholic and nonalcoholic fatty liver disease. Research has revealed mechanisms that underlie the initiation and progression of both alcoholic and nonalcoholic fatty liver disease, especially with respect to the pathways that control hepatic lipid metabolism. These studies may well lead to means of treating fatty liver disease that are based on physiological principles.

Pathogenesis of Fatty Liver Disease: There are several candidates for the "second hit" that is involved in the evolution from simple steatosis to steatohepatitis. One of the more compelling candidates is oxidative stress caused by reactive oxygen species (ROS), which have been shown to be increased in both alcoholic and nonalcoholic fatty liver disease. Furthermore, experimental evidence suggests that liver damage may be constrained, at least in part, by upregulation of antioxidant defenses and other survival responses. Other factors that appear to be important in the development of steatohepatitis are fatty acids, cytokines (e.g., adiponectin, leptin, and resistin from adipose tissue, and TNF-alpha and TGF-beta from Kupffer cells), and the metabolic activation of enzyme pathways in hepatocytes. Defining the roles of these factors, as well as the pathways in which they participate, in the natural history of alcoholic hepatitis, NASH, and the secondary forms of fatty liver disease will facilitate prevention and control of these diseases.

Diagnosis and Monitoring of Fatty Liver Disease: The diagnosis of steatohepatitis is still dependent upon liver biopsy. While many studies have focused upon identification of noninvasive clinical features, using serum markers and imaging tests that might discriminate between steatosis and steatohepatitis, these approaches have not proven very reliable. The natural history of alcoholic and nonalcoholic fatty liver disease has been defined to a limited degree. However, particularly in NASH, the factors that predict poor outcome and eventual development of cirrhosis are still not well understood.

Therapy of Fatty Liver Disease: Therapy for fatty liver disease is still not completely effective or satisfactory to patients. For alcoholic liver disease, abstinence remains the only effective therapy, except for the use of corticosteroids and perhaps pentoxifylline during severe, acute disease. Trials of antioxidants such as S-adenosylmethionine (SAMe) and anticytokines (anti-TNF) are under way. Clearly, therapies that speed recovery from alcoholic hepatitis and fatty liver disease, as well as treatments for alcohol dependence and abuse, would be of great benefit. NASH is often responsive to weight loss, although in practice this is very difficult to achieve and maintain in the long term. Several small, uncontrolled trials have suggested that therapy of NASH with insulin-sensitizing

agents such as metformin or the thiazolidinediones may improve biochemical and histological abnormalities. Promising results have also been obtained with diet and exercise therapy and with antioxidants and herbal medications (e.g., vitamin E, silymarin, SAMe, betaine). However, overall efficacy and the risks of long-term therapy with these agents will depend on further study before they can be recommended.

Evidence emerging from animal studies suggests that alcoholic and nonalcoholic fatty liver disease may be improved by similar pharmacotherapy. These findings complement laboratory and clinical evidence that these two diseases have common immunologic and molecular mechanisms. For these reasons, progress in understanding and treatment of one form of fatty liver disease is likely to benefit the other.

RESEARCH GOALS

The ultimate goals for research in fatty liver disease are to understand the basic mechanisms of injury and to develop means of prevention and treatment of nonalcoholic and alcoholic fatty liver disease.

Basic Research on Hepatic Lipid Metabolism: Advances in fatty liver disease research are fundamentally linked to improved understanding of the normal pathways of hepatic lipid uptake, synthesis, metabolism, and secretion, and how these pathways are deranged in fatty liver disease. There has been excellent progress made in the molecular delineation of the pathways of biosynthesis and metabolism of cholesterol, fatty acids, triglycerides, and other lipids, but the components of these pathways that are altered or defective in various types of fatty liver disease are not well defined.

- *Research Goal:* To perform an integrative analysis of normal and abnormal pathways of lipid metabolism in the liver and the compensatory changes that occur when these pathways are altered or defective (Matrix Cell B2; see also Chapter 1, A2).

There are currently several animal models of fatty liver disease, but none of them adequately reflects the pattern and progression of cell injury, inflammation, and fibrosis that are characteristic of NASH or alcoholic liver disease. Animal models for TPN-associated liver disease are also important and may provide insights into the pathogenesis of both alcoholic and nonalcoholic steatohepatitis. Analysis of the nature and cause of the inflammation and cell damage in these models might help elucidate the factors responsible for the progression of steatosis to steatohepatitis. Importantly, the development of reliable animal models requires clinical and molecular comparisons to human disease(s), which emphasizes the importance of complementing bench-to-bedside with bedside-to-bench research.

- *Research Goal:* To develop animal models of nonalcoholic liver disease that would allow better definition of molecular mechanisms (Matrix Cell A3).

Clinical Investigation of Pathogenesis: Current understanding of the factors that drive fatty liver disease progression from steatosis to steatohepatitis to cirrhosis is incomplete.

- *Research Goal:* To more fully characterize the clinical, metabolic, and molecular abnormalities present during the multiple stages of progression of nonalcoholic and alcoholic liver disease (Matrix Cell B1).

Such characterization could include proteomic, metabolomic, and gene expression array studies of well characterized patients with steatosis only, early steatohepatitis, steatohepatitis with fibrosis, and cirrhosis. Particular attention could be given to development of hepatocellular carcinoma in patients with NASH, including definition of overall frequency,

risk factors, and biomarkers. These types of investigations could provide clues as to which gene expression and protein levels are increased, decreased, or abnormal (e.g., in terms of glycosylation or cellular distribution) at the various stages of fatty liver disease. These results could then be compared to similar molecular analyses of animal models to identify and explore pathways that appear to be integral to the etiology of this liver disease. Comparisons could also be made between alcoholic and nonalcoholic liver disease, using these advanced molecular approaches, as well as standard clinical approaches.

One aim of these studies is to assess whether alcoholic liver disease is truly a separate entity from NASH, or, rather, represents a form of NASH in which the major caloric challenge is alcohol. Similarly, other forms of fatty liver disease (e.g., TPN-associated liver disease, drug-induced steatohepatitis, fatty liver from lipodystrophy) could be evaluated, compared, and contrasted to more typical forms of NASH. Another central issue is whether fatty liver disease is largely due to a single mechanism versus multiple mechanisms.

Another issue requiring additional study is that only a proportion of persons who drink alcohol excessively for decades develop alcoholic hepatitis or liver injury. Similarly, fatty liver is common in overweight or obese individuals, but only a proportion of these individuals develop NASH. Thus, some persons who drink more than 100 grams of alcohol daily for decades do not develop steatohepatitis, whereas some persons who drink as little as 40 to 50 grams daily for 5 to 10 years develop florid alcoholic liver disease. Similarly, NASH is common in patients with obesity, but some patients develop severe NASH with normal body mass index (BMI) or with modest weight gain (BMI 25-30), whereas others with severe obesity (BMI≥40) have mild hepatic steatosis without cell injury or evidence of NASH. These inter-individual variations indicate that genetic or unknown environmental confounding factors contribute to disease expression.

- *Research Goal:* To establish a cohort study to prospectively analyze the natural history of NASH and other forms of nonalcoholic fatty liver disease (Matrix Cell A1).

These studies could also attempt to identify environmental factors, particularly factors related to nutritional status and patterns of carbohydrate, fat, and protein intake that may affect the expression and progression of NASH.

- *Research Goal:* To identify important genetic risk factors of fatty liver disease through large-scale genomic studies in well characterized cohorts of patients, both with and without steatosis, NASH, and advanced liver disease (Matrix Cell C3).

It is also important that findings from such clinical studies be re-applied to basic laboratory investigation to help define the specific pathways that lead to steatosis and steatohepatitis and to identify potential therapeutic targets.

Similarly, a more careful analysis of alcohol intake, including both "excessive" and "moderate" levels of alcohol intake performed in cohorts of patients with NASH, could yield important information on this risk factor.

- *Research Goal:* To better define the safe level of alcohol intake in different populations, including the overweight and obese, minority populations, and patients with diabetes and chronic viral hepatitis or other forms of nonalcoholic liver disease (Matrix Cell C2).

The evolution of simple steatosis to steatohepatitis is frequently attributed to a "second hit," which has been variously attributed to oxidative stress, activation of metabolic enzymes, insulin resistance, free fatty acids, or pro-inflammatory cytokines produced by adipose tissue, macrophages, or the immune system. Studies to characterize the clinical and metabolic patterns associated with fatty liver disease could

focus on understanding the status of reactive oxygen and nitrogen species; activation of the cytochrome P450 system; state of insulin resistance and activity of insulin signaling pathways; composition of lipids and free fatty acids in serum and liver; role of visceral adiposity; and status of cytokines and anti-cytokines in both animal models and humans with fatty liver disease. These studies should employ state-of-the-art methods to assess gene expression and protein production and metabolic patterns (i.e., proteomics and metabolomics). An important focus of these studies is the possibility that they will reveal targets for therapy of nonalcoholic and alcoholic liver disease.

Diagnosis and Monitoring: One of the roadblocks to therapeutic studies in alcoholic and nonalcoholic fatty liver disease is the need to perform liver biopsies to prove the existence of steatohepatitis. In fatty liver disease, serum aminotransferase (ALT and AST) levels are relatively unreliable in predicting the presence and severity of disease activity. Furthermore, imaging methods such as ultrasound, CT, and MRI are capable of detecting fat in the liver, but cannot reliably distinguish simple steatosis from steatohepatitis. The combination of proteomic and metabolomic analyses of serum from patients with various stages of fatty liver disease could provide biomarkers for disease severity and stage.

- *Research Goal:* To develop reliable noninvasive markers to distinguish simple steatosis from steatohepatitis and to assess disease activity (grade) and fibrosis (stage) (Matrix Cell B2; see also: Chapter 2, A3; Chapter 6, C2; Chapter 16, C1).

These noninvasive markers would greatly enhance the ability to make the diagnosis of fatty liver disease and evaluate therapy. These biomarkers might also help to define whether cases of cryptogenic cirrhosis are due to NASH. Such markers could also provide a way to estimate the prevalence of NASH in the general population, as well as in high-risk groups.

- *Research Goal:* To utilize newly developed noninvasive markers as means to screen populations for NASH and assess its prevalence (Matrix Cell C2).

Therapy and Prevention: Development of specific treatments for both forms of fatty liver disease is an important research goal.

- *Research Goal:* To identify targets for therapy that might be used in rapid-throughput screening systems based on findings *in vitro* and in animal models (Matrix Cell B3).
- *Research Goal:* To rapidly evaluate potential therapies suggested by animal studies or clinical investigation in well designed phase I and II clinical trials (Matrix Cell A2).

Several currently available agents have shown promise as therapies for fatty liver disease in small, open-labeled studies. These agents include metformin, the thiazolidinediones, vitamin E, silymarin, and anti-cytokines such as TNF receptor antagonists.

- *Research Goal:* To evaluate those available agents that demonstrate the most promise for treating fatty liver disease in adequately-powered, prospective, randomized controlled trials (Matrix Cell C1).

Such trials would be best conducted by including the full spectrum of disease observed in patients, including children. Prospective studies are also appropriate in TPN-associated liver disease with analysis of risk factors, specific elements in nutritional support, and means of prevention and treatment. Agents found to be promising in animal models and clinical trials in alcoholic liver disease and NASH deserve evaluation in TPN-associated fatty liver disease as well.

Additionally, the identification of risk factors for disease progression could help to determine which patients should receive therapy. Steatohepatitis

appears to be a slowly progressive condition that may require many years or decades of treatment to prevent progression to cirrhosis. The safest and least invasive approaches to therapy deserve the highest priority in evaluation, and long-term outcomes also warrant evaluation. In this respect, evaluation of dietary approaches to fatty liver disease is important, if for no other reason than to define which approaches (e.g., low-fat vs. high saturated fat vs. medium chain triglycerides, animal protein vs. vegetarian protein diets, supplementation with dietary anti-oxidants) are not harmful. Long-term studies of weight loss or weight management with exercise or lifestyle changes as therapy for NASH are also worthwhile. Alternatively, studies of weight-loss therapies for obesity might be expanded to include analysis of liver disease in these cohorts. In this regard, important groups to study are patients with severe obesity who are undergoing bariatric surgery. It is unclear whether bariatric surgery is beneficial or harmful to patients who have NASH in addition to severe obesity. Therefore, prospective studies of the effects of surgery on the existing liver disease are important.

- *Research Goal:* To assess the effects of bariatric surgery on progression of NASH (Matrix Cell B1).

In the case of fatty liver disease associated with excessive alcohol intake, new therapies to speed recovery and decrease permanent injury would benefit patients. Only through a balanced and coordinated program of clinical studies will such treatments be adequately evaluated for long-term safety and efficacy. Medications of promise for alcoholic liver disease need to be pursued, particularly those that are safe and well-tolerated.

- *Research Goal:* To develop therapy for acute alcoholic hepatitis (Matrix Cell B3).
- *Research Goals:* To establish safety and efficacy of promising medications for alcoholic liver disease through phase I and II trials, and progress to definitive phase III studies in agents of greatest promise (Matrix Cells A2 and C1).

Once there is a better understanding of the pathogenesis of fatty liver disease and means of treatment, it may be reasonable to start screening programs for this condition and to intervene with means of prevention or early treatment in high-risk groups (e.g., children who are obese or have type 2 diabetes).

- *Research Goal:* To develop screening and intervention programs for the early detection, prevention, and treatment of fatty liver disease (Matrix Cell C3).

STEPS TO ACHIEVE RESEARCH GOALS

Basic research on fatty liver disease would benefit from a closer coordination of research programs in alcoholic and nonalcoholic liver disease. There is much overlap in basic research performed on the two forms of fatty liver disease in terms of basic biology, animal models, important molecular pathways, potential targets for screening therapies, and communities of investigators. Coordination of these communities and resources can be facilitated by the many NIH Institutes that fund research on fatty liver disease, as well as through integration of grant application review by the Center for Scientific Review at the NIH. Further coordination among Federal agencies, including the NIH, Department of Veterans Affairs, and Centers for Disease Control and Prevention, would be beneficial in this regard. Many of the clinical research goals identified in fatty liver disease would benefit from creation of coordinated, large, prospective cohort studies on alcoholic liver disease, NASH, and secondary forms of fatty liver disease. These studies could also include (or might focus largely upon) prospective clinical trials of promising agents for fatty liver disease. These elements are well served by the ongoing NASH Clinical

Research Network funded jointly by the NIDDK and NICHD, which has included both adult and pediatric cases and has initiated two randomized controlled trials. In a similar manner, the recently established Longitudinal Assessment of Bariatric Surgery (LABS) Consortium provides an excellent opportunity to study the natural history and risk factors for NASH in severely obese subjects and the effects of bariatric surgery on the liver. It is important that these cohort studies and trials include ancillary studies focusing on pathogenesis and delineation of the mechanisms of action of the agents under investigation, using state-of-the-art molecular approaches to clinical, genetic, metabolomic, and biochemical investigation.

Similarly, in alcoholic liver disease, the large, prospective clinical trial of SAMe recently initiated by the Department of Veterans Affairs will focus on safety and efficacy of the therapy, and will also provide means of molecular and genetic analyses in a well characterized and well followed cohort of patients. Other mechanisms and innovative approaches would help to facilitate phase I and II clinical trials in alcoholic and nonalcoholic liver disease. These approaches could engage the multiple NIH Institutes and Federal agencies involved in research on fatty liver disease and could also reach out to industry to engage them in developing means to prevent and control fatty liver disease.

Matrix of Research Goals in Fatty Liver Disease

	Short Term (0-3 years)	Intermediate Term (4-6 years)	Long Term (7-10 years)
High Risk	**A3.** Develop more accurate animal models of nonalcoholic fatty liver disease (including secondary forms) and define molecular characteristics.	**B3.** Develop rapid-throughput systems to evaluate potential therapies of fatty liver disease. Develop therapy of acute alcoholic hepatitis that promotes recovery and decreases permanent injury.	**C3.** Identify genetic markers for development of steatohepatitis and its complications. Develop screening programs for early detection and intervention with preventive or therapeutic regimens.
Intermediate Risk	**A2.** Conduct phase I and II clinical trials of candidate therapies for NASH, TPN-associated liver disease, and alcoholic liver disease (e.g., silymarin, cytokines, anti-cytokines, anti-fibrotic agents).	**B2.** Delineate the hepatic pathways of lipid metabolism and how they are altered in alcoholic and nonalcoholic liver disease. Develop noninvasive means of distinguishing steatosis from steatohepatitis and for grading and staging disease.	C2. Establish the prevalence and incidence of NASH in the general population as well as special populations in the United States, such as children, minority groups, and patients with diabetes and other dysmetabolic syndromes. Better define the safe amounts of alcohol intake in terms of liver disease for different populations.
Low Risk	**A1.** Establish cohort study to prospectively analyze the natural history of the full spectrum of nonalcoholic fatty liver disease.	**B1.** Elucidate the clinical, metabolic, proteomic, and gene expression patterns associated with various stages of nonalcoholic and alcoholic fatty liver disease. Evaluate role and effects of bariatric surgery on NASH.	**C1.** Establish the efficacy and safety of therapy with insulin-sensitizing agents and vitamin E in NASH. Establish the efficacy and safety of therapy with SAMe in ALD.